Infection of Mice with *Neospora caninum* (Protozoa: Apicomplexa) Does Not Protect against Challenge with *Toxoplasma gondii*†

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*Neospora caninum* and *Toxoplasma gondii* are structurally related protozoal parasites of mammals that may cause abortion and neonatal morbidity and mortality. Groups of mice were subcutaneously inoculated with 10⁶ live zoites of the NC-1 or NC-3 isolates of *N. caninum* and reinoculated with an identical number of live zoites 2 weeks later. Groups of mice which were injected subcutaneously with Hanks balanced salt solution served as controls. Three weeks after the final *N. caninum* inoculation, one-half of the mice were inoculated subcutaneously with 2.5 × 10⁴ zoites of the RH isolate of *T. gondii* and the other half were inoculated subcutaneously with 2.5 × 10⁴ zoites of the GT-1 isolate of *T. gondii*. Serum samples taken from mice on the day of *T. gondii* inoculation were negative for specific antibodies to *T. gondii*, but mice inoculated with *N. caninum* had reciprocal titers of ≈800 to this protozoan. All of the mice died after challenge with *T. gondii*, and no significant differences (*P > 0.05*) between the survival times of mice inoculated with either isolate of *N. caninum* and those of control mice were seen. This study indicates that *N. caninum* and *T. gondii* are distinct biologic entities and not closely related isolates.

*Neospora caninum* is a newly recognized protozoan parasite that has been confused with and misdiagnosed as *Toxoplasma gondii* (5). *N. caninum* causes severe disease and death in transplacentally infected dogs (1, 2, 5, 8). The disease in these dogs is characterized by polyradiculoneuritis, encephalitis, pneumonitis, and ascending paralysis (2, 8). A mother dog may transmit infection to several successive litters (8). In addition to infections in dogs, abortions in cattle (18) and neonatal paralysis in calves and lambs have been documented (7, 9, 12). Experimental studies have demonstrated that transplacental infections occur in dogs and cats (10, 11) and abortions occur in sheep (11a). Tissue cysts from the brains of mice and zoites from cell cultures can produce infections in mice (16, 17). Other aspects of the life cycle and transmission of *N. caninum* are not known; however, infections in ruminants suggest that an oocyst stage is present in an unidentified definitive host. It is not known whether humans can be infected with *N. caninum*, but the wide variety of mammals that have recently been shown to be infected suggests that humans are at risk.

*Hammondia hammondi* is a protozoan parasite of cats and other mammals that is structurally and antigenically related to *T. gondii* (13). It is well documented that active infection with *H. hammondi* protects against challenge inoculation with *T. gondii* (3, 4, 13). The present study was undertaken to determine whether infection with *N. caninum* would protect mice against a challenge inoculation with *T. gondii* and thereby better define the relationship between these two protozoan parasites of mammals.

NC-1 and NC-3 isolates of *N. caninum* and RH and GT-1 isolates of *T. gondii* were used in this study. The zoites used for inoculations were grown in and harvested from bovine monocye cell cultures as previously described (15). Infection of mice with either *N. caninum* isolate usually causes no clinical signs of disease, while infection with either isolate of *T. gondii* causes fatal infection.

Two identical experiments were done, and the data were combined (see Table 1). Outbred female Swiss Webster mice (body weight, 20 to 25 g) were used. Groups of mice (10 per group [total]) were inoculated subcutaneously with 10⁵ live zoites of either the NC-1 (groups 1 and 2) or the NC-3 (groups 3 and 4) isolate and then reinoculated with an identical number of homologous live zoites 2 weeks later. Control mice (groups 5 and 6) received Hanks balanced salt solution subcutaneously on those occasions. Three weeks after the final *N. caninum* inoculation, serum samples were collected from each mouse and the mice in groups 1, 3, and 5 were subcutaneously inoculated with 2.5 × 10⁵ live zoites of the RH isolate and the remaining mice (groups 2, 4, and 6) were subcutaneously inoculated with an identical number of live zoites of the GT-1 isolate.

Serum from mice were examined for immunoglobulin G antibodies to *N. caninum* and *T. gondii* by an indirect immunofluorescent assay (16). Zoites of the NC-1 isolate of *N. caninum* and the RH isolate of *T. gondii* were used as the antigen. Sera were examined at twofold dilutions from 1:50 to 1:800. Additionally, sera were examined for antibodies to *T. gondii* in a direct agglutination assay using Formalin-fixed RH isolate zoites as the antigen (6). Sera were examined at dilutions of 1:50, 1:100, and 1:500 in the agglutination test. Impression smears were made from the livers and/or lungs of mice after death and examined for parasites.

Mean days of survival after *T. gondii* inoculation were analyzed by using nonparametric Kruskal-Wallis analysis of variance. Multiple contrasts were made by using Tukey-like comparisons of Wilcoxon rank sums. Designation of significant differences between means was based on a cutoff of *P < 0.05*.

Two mice (one from group 1 and one from group 2) inoculated with the NC-1 isolate of *N. caninum* developed neurological signs that consisted of moderate head tilting. The neurological signs were seen 19 days after the original

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TABLE 1. Survival of mice inoculated with Neospora caninum and challenged with Toxoplasma gondii

<table>
<thead>
<tr>
<th>Group</th>
<th>Neospora isolate</th>
<th>Toxoplasma challenge</th>
<th>Mean survival time (days) ± SEM (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NC-1</td>
<td>RH</td>
<td>8.1 ± 0.10 (8–9)</td>
</tr>
<tr>
<td>2</td>
<td>NC-1</td>
<td>GT-1</td>
<td>11.2 ± 0.85 (8–14)</td>
</tr>
<tr>
<td>3</td>
<td>NC-3</td>
<td>RH</td>
<td>7.9 ± 0.30 (8–11)</td>
</tr>
<tr>
<td>4</td>
<td>NC-3</td>
<td>GT-1</td>
<td>10.5 ± 0.78 (8–13)</td>
</tr>
<tr>
<td>5</td>
<td>None</td>
<td>RH</td>
<td>7.6 ± 0.16 (7–8)</td>
</tr>
<tr>
<td>6</td>
<td>None</td>
<td>GT-1</td>
<td>9.2 ± 0.50 (7–11)</td>
</tr>
</tbody>
</table>

* There were 10 mice per group, except in group 2, which had 9 mice.

* Not statistically different (P > 0.05).

* Control mice were subcutaneously inoculated with Hank's balanced salt solution.

NC-1 inoculation. A mouse in group 2 was found dead 14 days after the original NC-1 inoculation. A necropsy was done, and no parasites were seen in the liver, lungs, or brain of that mouse. Because the cause of death was not related to the inoculated parasites, that mouse was not considered in the statistical analysis of data. None of the mice inoculated with the NC-3 isolate (groups 3 and 4) or the control mice (groups 5 and 6) developed clinical signs of disease before inoculation with T. gondii.

All of the mice died after challenge with T. gondii. Mice inoculated with either isolate of N. caninum lived longer than the controls; however, this increased survival time was not significant (P > 0.05) (Table 1). Mice inoculated with the RH isolate died sooner than mice inoculated with the GT-1 isolate. Gross lesions of hepatitis and pneumonia were present in mice in the three groups inoculated with the RH isolate, while the primary gross lesion seen in mice in the three groups inoculated with the GT-1 isolate was pneumonia. Zoites were present in the livers or lungs of all mice. Reciprocal titers of antibody to N. caninum of ≥800 were present in the sera of mice in groups 1 to 4. No antibodies to N. caninum were found in control mice from groups 5 and 6. None of the mice had detectable antibodies to T. gondii in their sera.

This study demonstrated that mice infected with N. caninum were not protected against a lethal challenge with T. gondii. The absence of specific T. gondii antibodies in mice inoculated twice with living N. caninum zoites indicates that cross-reactive antibodies were not formed following active infection. Although mice inoculated with N. caninum in the present study did not have significantly prolonged survival time, they did survive longer than controls. This might have been due to stimulation of nonspecific cell-mediated immune factors by the N. caninum infections. It has been reported that cell-mediated immunity is more important in protection against T. gondii infection than is antibody (14).

The results of this study demonstrate that the two parasites are separate entities and that the relationship between N. caninum and T. gondii is different from that which exists between T. gondii and Hammondia hammondi.